Improved PCR flexibility with Hot Start dNTPs

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Introduction

Considered one of the most powerful tools in molecular biology, PCR is a well-used technique for the amplification of DNA fragments of interest. While ubiquitous, PCR is not flawless. Reactions are often handicapped by nonspecific amplifications such as primer dimerization and mis-priming (1). These off-target amplifications result from primer interaction and extension at lower, less-stringent temperatures and can significantly reduce the efficiency of PCR by inhibiting the amplification of the desired target (2).

There have been numerous advancements in the field of PCR that aim to overcome such off-target amplifications—one approach is termed Hot Start. In this technique, primer extension is blocked until higher thermocycling temperatures are reached. Many technologies have evolved since the inception of Hot Start including methods that physically separate reaction components, inhibit DNA polymerase, employ accessory proteins, and utilize chemically modified primers. While these advancements are innovative and useful, they can drive up the cost of PCR considerably. This presents a dilemma between sacrificing an efficient and robust result for a lower cost reaction. One approach that was not explored yet was chemically modifying the dNTP component of the PCR mix. Since dNTPs are an essential part of all PCR reactions, modified dNTPs could easily replace standard dNTPs without the need to significantly alter existing PCR protocols.

CleanAmp™ dNTPs contain a thermolabile tetrahydrofuranyl (THF) protecting group that allows for Hot Start activation in PCR and results in improved PCR performance (3). At the lower temperatures of PCR setup, the 3'-THF dNTP blocks primer extension and prevents primer dimerization and mis-priming. When the temperature of the reaction is elevated to higher temperatures, the protecting group is released. This forms the standard dNTP, which now becomes an available DNA polymerase substrate. Herein, the versatility and utility of CleanAmp™ dNTPs as compared to standard dNTPs are investigated.

Materials and methods

PCR protocols for the annealing temperature gradient experiment (Figure 1, A and B) consisted of 1× PCR buffer (20 mM Tris pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$; Invitrogen), 1.25 U Taq DNA polymerase (Invitrogen), 0.2 μM primers (TriLink BioTechnologies), 0.2 mM dNTPs (standard dNTPs, New England Biolabs; CleanAmp™ dNTPs, TriLink BioTechnologies), and 1 ng Human Genomic DNA as template (Promega). Each 25-μL reaction was conducted

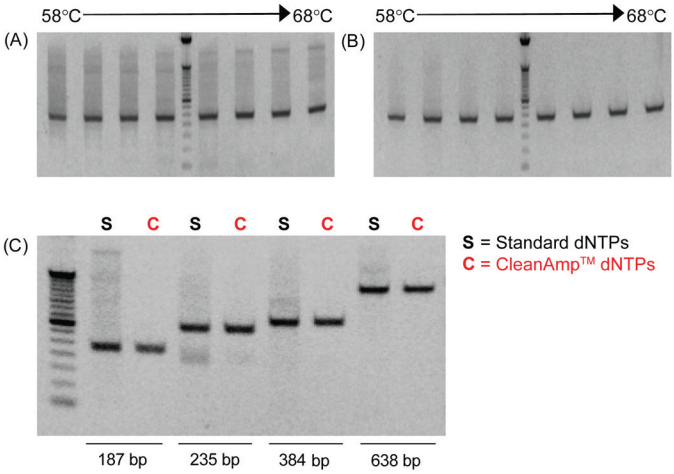

Figure 1. Comparison of (A) standard dNTPs and (B) CleanAmp™ dNTPs for amplification of a 235 bp target from human genomic DNA over a range of annealing temperatures. (C) Performance of standard and CleanAmp™ dNTPs for the amplification of four targets from human genomic DNA.

in a single, thin-walled 200-μL tube and placed in a BioRad Tetrad 2 thermal cycler with a thermal cycling protocol of 95°C (10 min); [95°C (15 s), 58-68°C (30 s), 72°C (1 min)] 35×; 72°C (5 min). After PCR, 20 μL each sample was electrophoresed in a 2% agarose E-gel (Invitrogen) and visualized using an Alpha Innotech Corporation Multi Image Light Cabinet with CCD Camera. The primer sequences employed in studies were specific for formation of a 187 bp (5' AACCAACCAGATGTGT TCCGTGTCC, 5' CCTGCAAGACCACCACCACAATCG), 235 bp (5' CCCTGGACT TCGAGCAAGAGATGG, 5' GAGGGAAATGAGGGCAGGACTTAGC), 384 bp (5' AGGAGGTGGGAAGGGACTATTTGG, 5' GTCTCCAGTCACAAGGCAGAATCC), and a 638 bp (5' TGCTGGGTGGTGGTCATCTTTCC, 5' CCGTCCGTTGTATGTCTGCTATGC) amplicon.

For the experiment comparing CleanAmp™ dNTPs to standard dNTPs using targets of different lengths (Figure 1C), reaction conditions were similar to those listed above, with a specific annealing temperature of 63°C. When the CleanAmp™ dNTPs were compared to the standard dNTPs in quantitative PCR, reaction conditions were also similar to the previous two experiments with the addition of varying template concentrations (0.01–100 ng human genomic DNA), 0.75 μM ROX reference dye, and 0.1 μM hydrolysis probe (5' HEX-CATCACCCGATGCTCGTGTTCATCAAAGT-

BHQ1). Reactions, which amplified the 187 bp target, were set up in triplicate and samples were run on a Stratagene MX3005P™.

Results and Conclusion

Implementation of new PCR protocols or modifications to current protocols often requires thermal cycling optimization experiments for robust PCR performance. To determine the optimal thermal cycling conditions for amplification of a 235 bp target from human genomic DNA, reactions containing standard and CleanAmp™ dNTPs were tested at different annealing temperatures ranging from 58°C to 68°C. At each annealing temperature, CleanAmp™ dNTPs improved reaction specificity as mis-priming was eliminated or significantly reduced relative to standard dNTPs. Furthermore, target amplicon yields were comparable across the entire annealing temperature range when CleanAmp™ dNTPs were employed (Figure 1, A and B). These results highlight the versatility of CleanAmp™ dNTPs, which removes the need to conduct extensive optimization experiments. After evaluating the utility of CleanAmp™ dNTPs at different annealing temperatures, their ability to amplify targets of different lengths from human genomic DNA was investigated. Results revealed that the use of CleanAmp™ dNTPs successfully amplified each of the desired targets, while drastically reducing or eliminating the mis-priming artifacts that occurred when standard dNTPs were used (Figure 1C).

In a final set of experiments, the CleanAmp™ dNTP technology was evaluated in quantitative PCR. Using hydrolysis probe detection, a 187 bp target from human genomic DNA was amplified with standard or CleanAmp™ dNTPs over a template range of 0.01–100 ng (Figure 2). When standard dNTPs were employed, the Cq (quantification cycle) values were delayed when compared to the corresponding reactions using CleanAmp™ dNTPs (Figure 2A). Moreover, the amplification curves for PCR experiments using CleanAmp™ dNTPs had a robust log phase of amplification, which is indicative of a higher level of PCR efficiency (4). In contrast reactions employing standard dNTPs had a shorter log phase of amplification and reached a lower fluorescence plateau. When the resultant data were plotted in a standard curve (Figure 2B), CleanAmp™ dNTPs successfully amplified each template concentration and displayed a higher efficiency than reactions with standard dNTPs. These findings demonstrate the increased PCR sensitivity when CleanAmp™ dNTPs are employed as the limit of detection is improved by an order of magnitude.

Herein, we present the further development of Hot Start dNTPs (CleanAmp™ dNTPs) for improved PCR performance (3), which allow the end user great flexibility. CleanAmp™ dNTPs provide benefit over a broad range of annealing temperatures, thereby minimizing optimization time and avoiding major changes to existing PCR protocols. In addition, CleanAmp™ dNTPs enhance PCR performance with targets of varying lengths and increase the efficiency and sensitivity of PCR, as shown in quantitative PCR. Overall, CleanAmp™ dNTPs are a novel, cost-effective route to Hot Start PCR that improves standard PCR protocols and can function under a wide range of conditions. Learn more at www.trilinkbiotech.com/cleanamp/dntps.

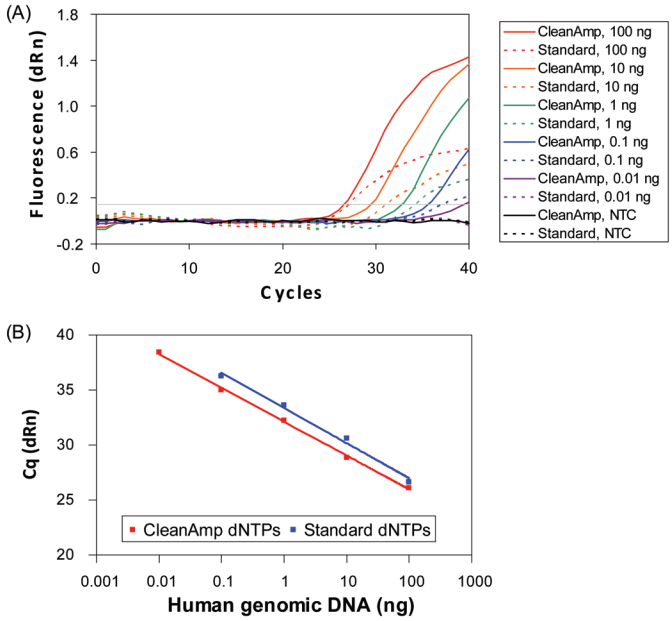

Figure 2. Comparison between standard dNTPs and CleanAmp™ dNTPs for amplification of a 187 bp target from human genomic DNA in quantitative PCR. Results are presented as an amplification plot (A) and a standard curve (B) with standard dNTPs: (Y = -3.191*LOG(X) + 33.36, Eff. = 105.8%) and CleanAmp™ dNTPs (Y = -3.079*LOG(X) + 32.12, Eff. = 111.2%).

References

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